Supplementary Information for

In vivo imaging of invasive aspergillosis with ^{18}F-fluorodeoxysorbitol positron emission tomography

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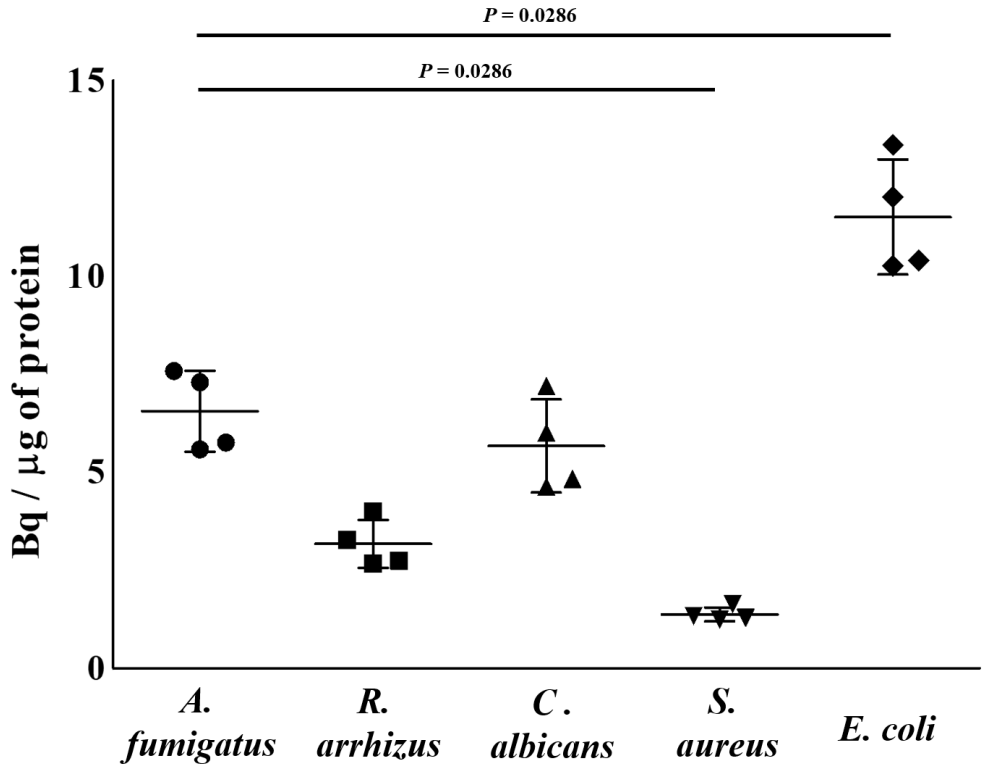
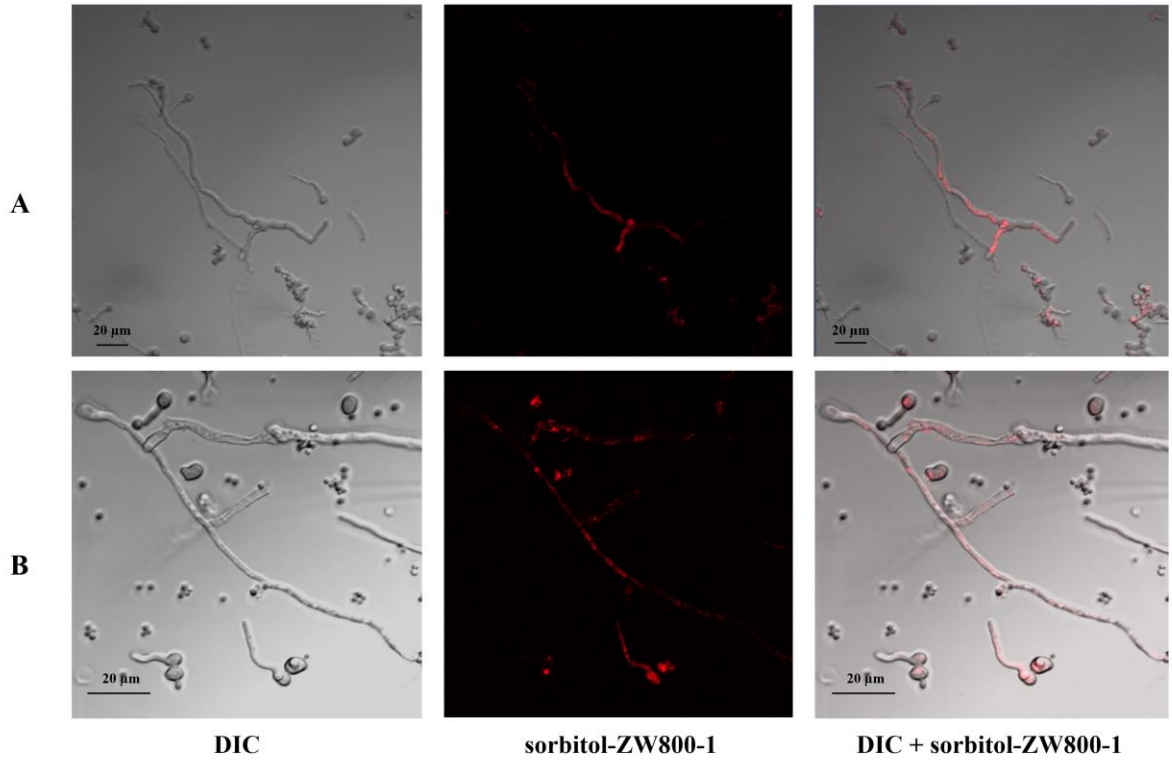
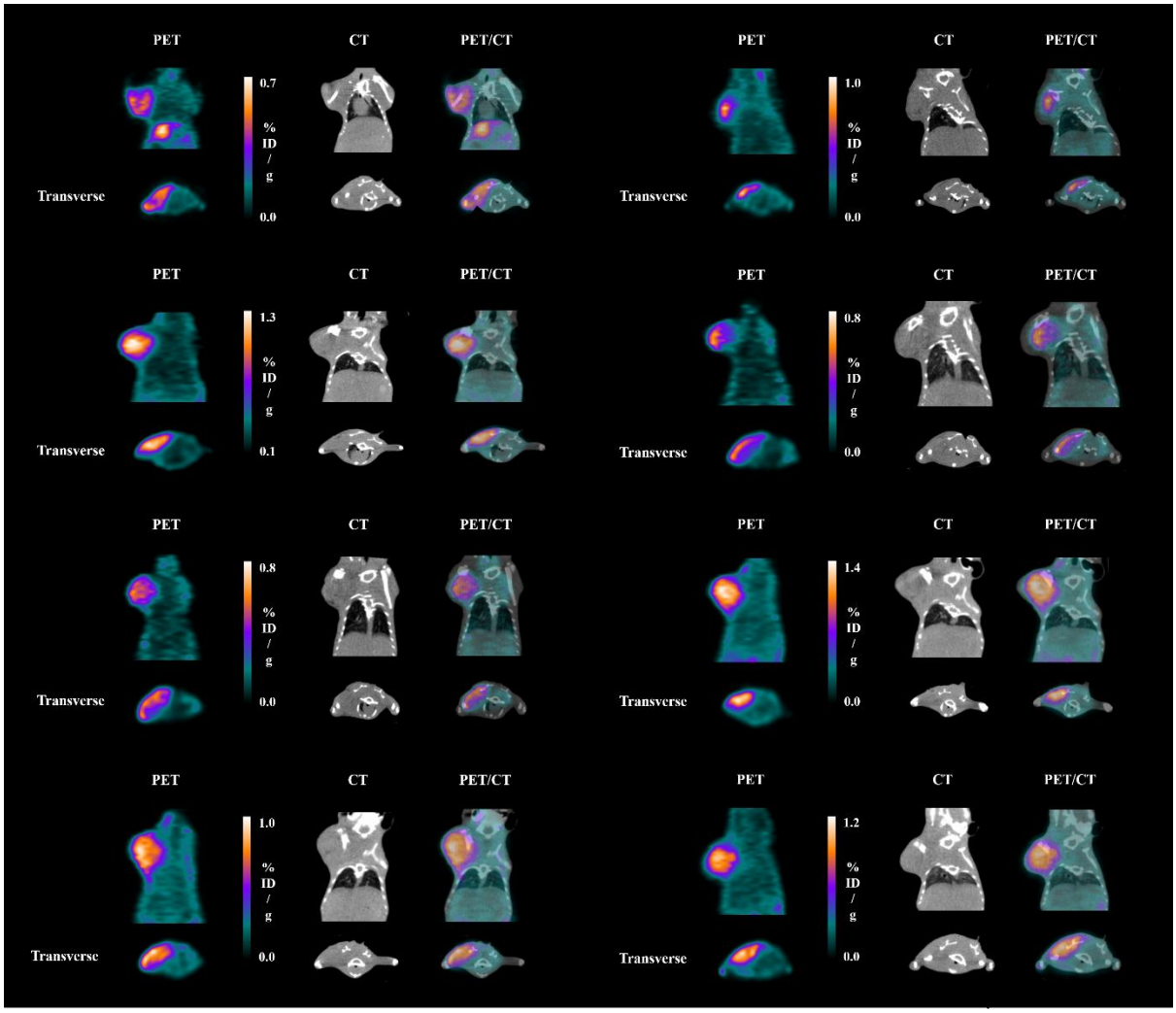


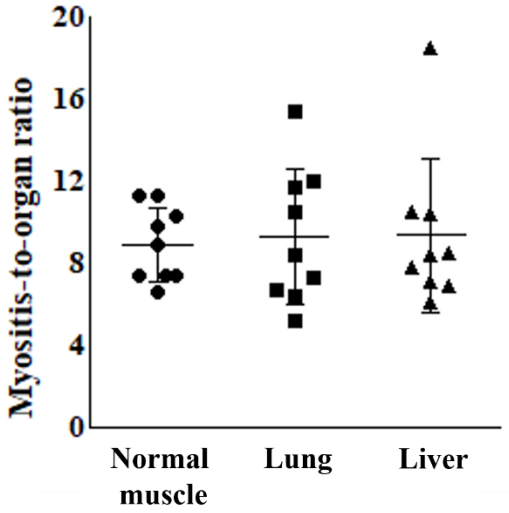
Supplementary Figure 1. Cellular uptake of ^{18}F-FDS by *A. fumigatus*, *R. arrhizus*, *C. albicans*, *S. aureus* (−), and *E. coli* (+) cells 1 h after treatment with ^{18}F-FDS. Data are expressed as the mean of absolute accumulation activity (Bq) ± SD (normalized by protein of 1 × 10^6 cells) of four replicate experiments ($^*P < 0.05$). Statistical significance was calculated using two-tailed Mann-Whitney U tests.



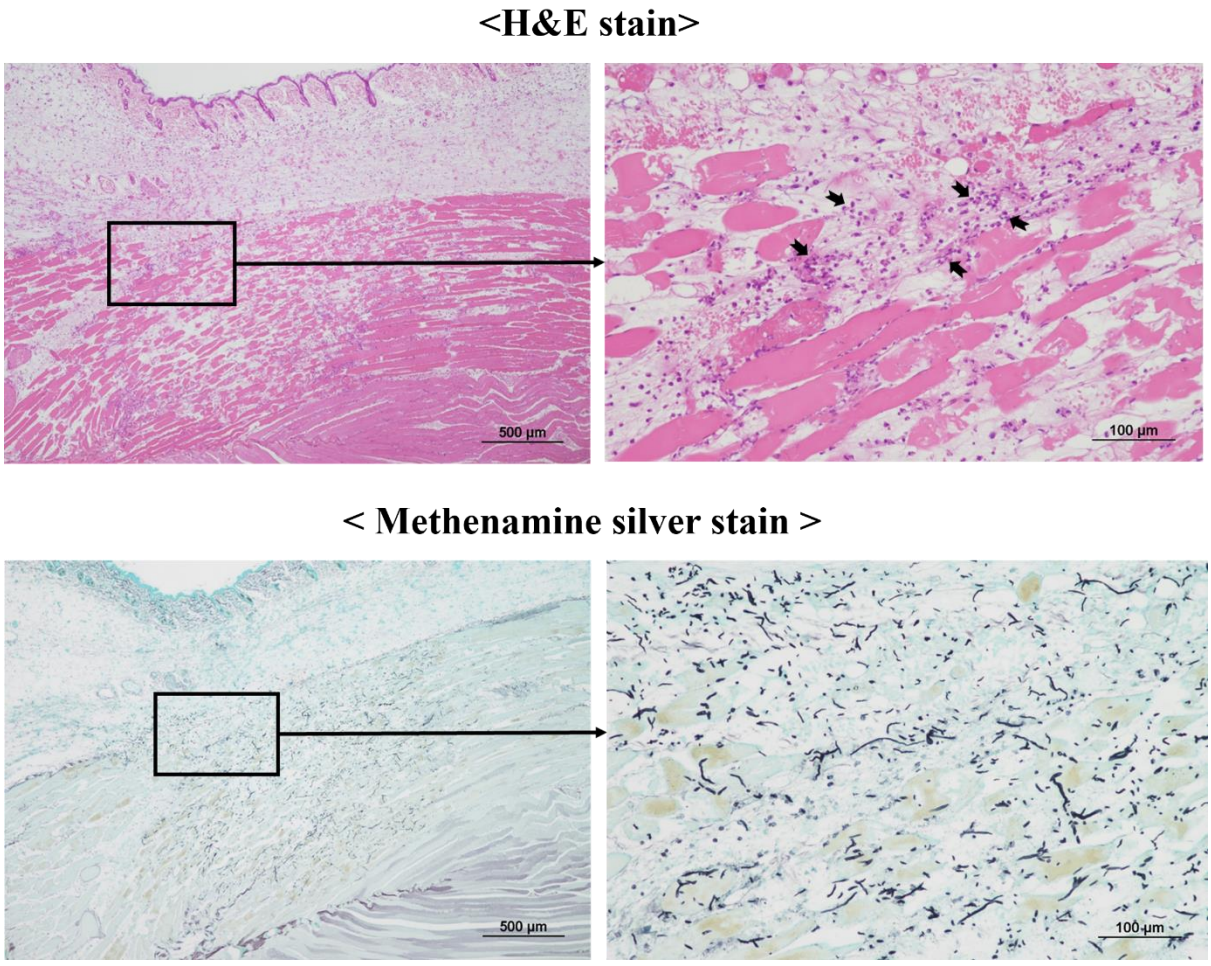
Supplementary Figure 2. Confocal microscopy demonstrates live binding of sorbitol-ZW800-1 to different growth stages of *A. fumigatus*. *A. fumigatus* was cultured on potato dextrose agar plates for 5–7 days at 30℃ and colonies were harvested using saline containing 0.1% (v/v) Tween 20. The suspensions were vortexed to release the conidia and filtered through a 40 μm cell strainer to remove clumps and hyphae. Conidia were counted using a hemocytometer, and 1×10^7 *A. fumigatus* conidia and 50 μM of sorbitol-ZW800-1 were co-cultured for up to 48 h in 1 mL of YPD broth in 24-well plates at 37℃ without agitation. Co-cultured *A. fumigatus* were observed at 20 and 48 h using laser scanning confocal microscopy (LSM 800, ZEISS, Germany). Before microscopic examination, cultured *A. fumigatus* were washed twice, re-suspended in PBS, and transferred to a confocal dish. Fluorescence of ZW800-1 (red) was obtained using laser filter excitation at 561 nm and emission at 650–750 nm. Image acquisition and analysis were performed using ZEN 2.6, blue edition (ZEISS, Germany). Merged differential interference contrast (DIC) and laser scanning images show more sorbitol accumulated in germinated *A. fumigatus* than in conidia. (A) Twenty hours of incubation × 200, n = 3; (B) forty-eight hours incubation × 400, n = 3.



Supplementary Figure 3. MicroPET, CT, and PET/CT fusion images of ^{18}F-FDS 2 h after injection (7.4 MBq) in mice with *A. fumigatus*-infected myositis.

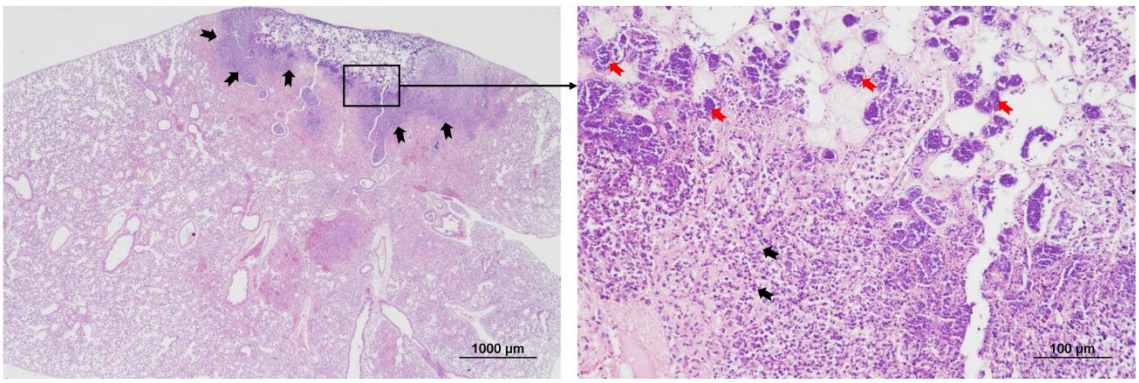


Supplementary Figure 4. *A. fumigatus* conidia to organ uptake ratio of ^{18}F-FDS in mice with *A. fumigatus*-infected myositis 2 h after injection (7.4 MBq, n = 9, each, Data are expressed as the mean ± SD).

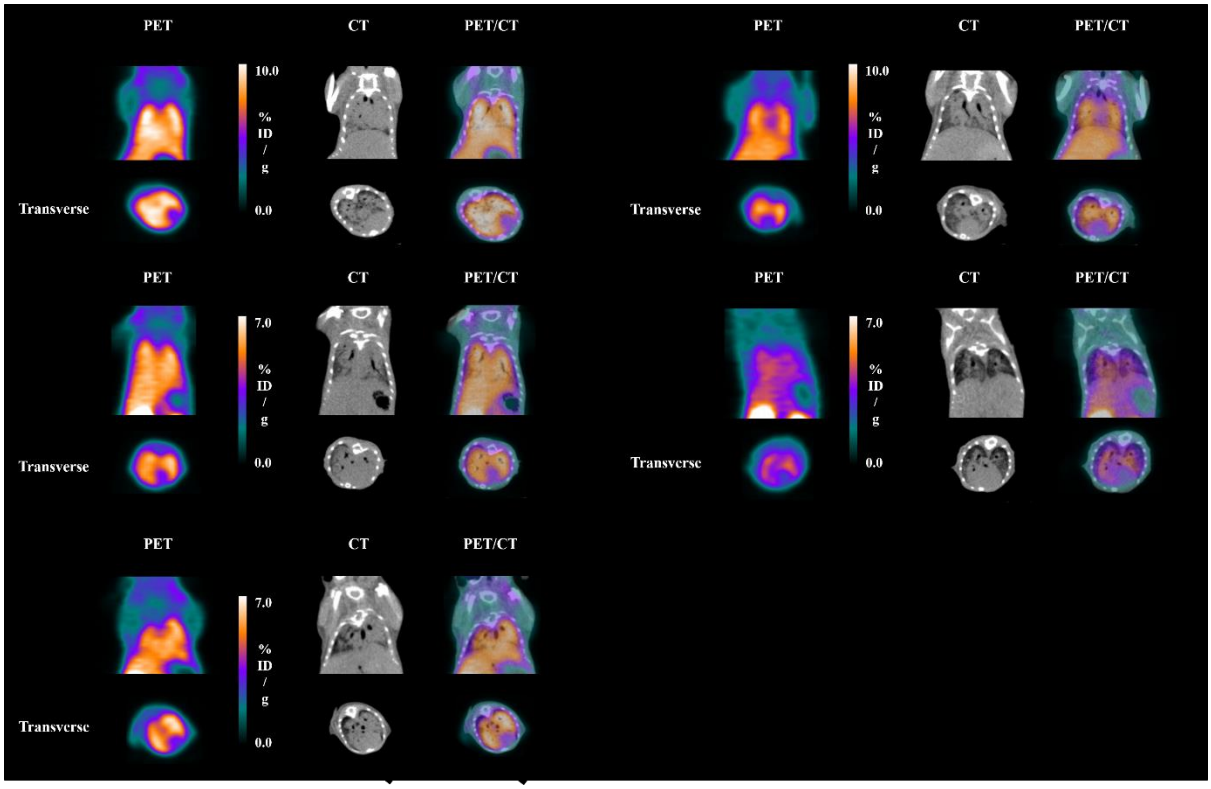


Supplementary Figure 5. Pathological examination of a mouse with *A. fumigatus*-infected myositis (n = 4). (Left upper) Hematoxylin and eosin (H&E) staining showing the destruction of muscle fibers and infiltration of inflammatory cells into muscle fibers (original magnification × 40). (Right upper). Magnified view of the box in the left upper figure (original magnification × 200). (Left lower) Methenamine silver staining showing scattered hyphae of *A. fumigatus* infiltrating into muscle fibers (original magnification × 40). (Right lower) Magnified view of the box in the left lower figure (original magnification × 200).

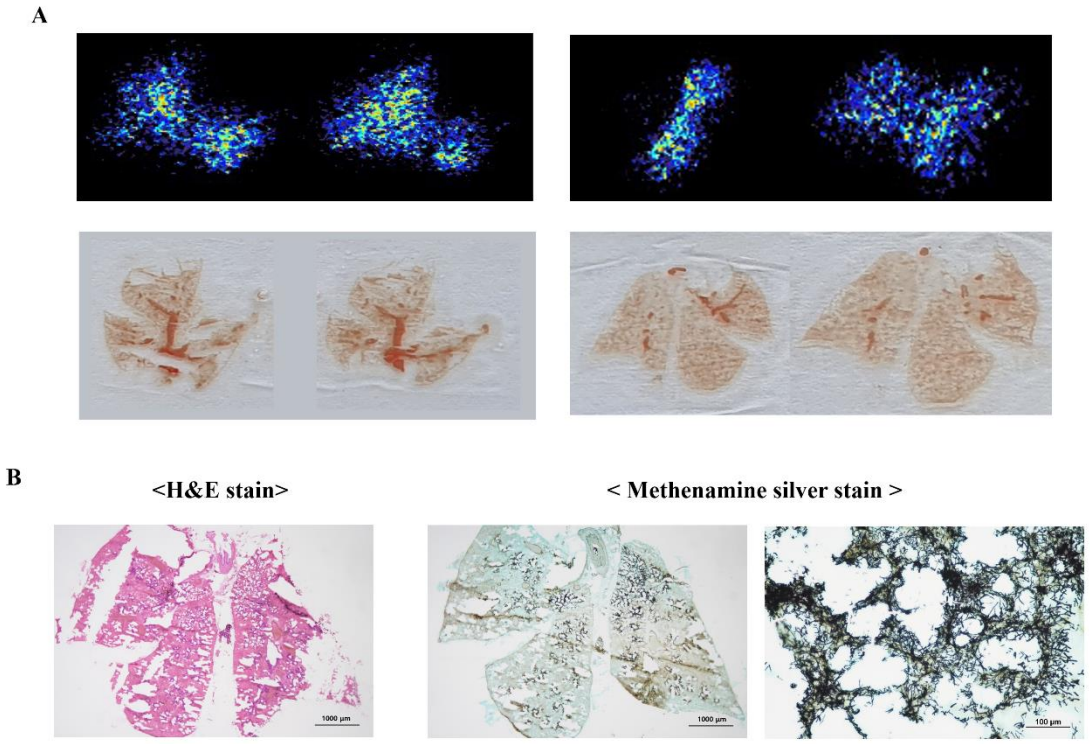
<H&E stain>



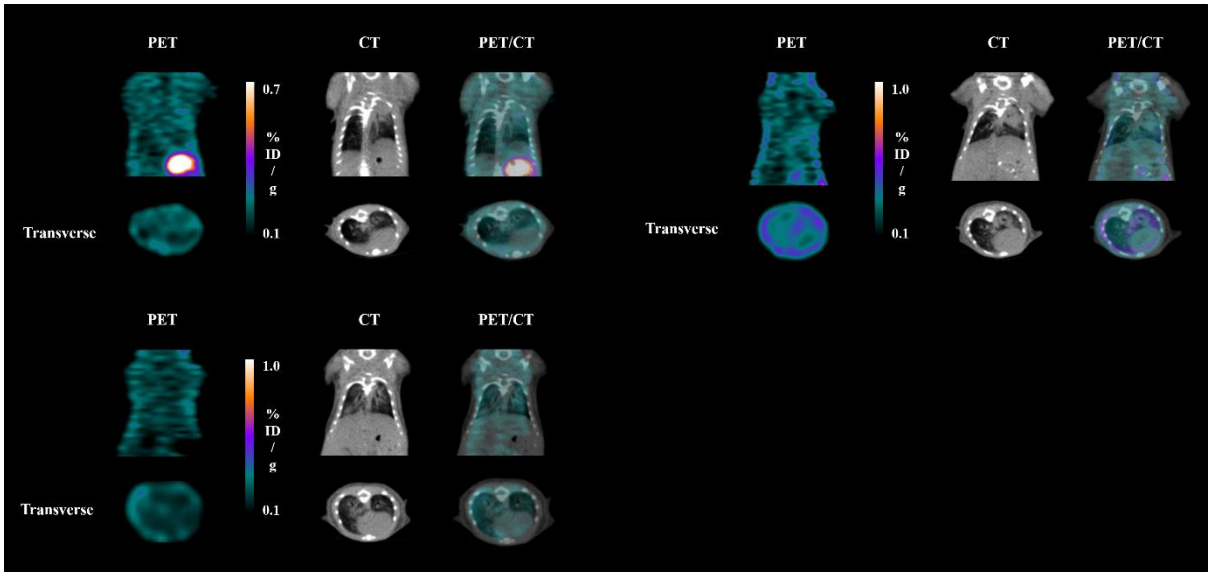
Supplementary Figure 6. Pathological examination of a mouse with *S. aureus*-infected lung tissue (n = 4). (Left side) Hematoxylin and eosin (H&E) staining, showing abscess formation and massive infiltration of inflammatory cells (arrows, original magnification × 20). (Right side) Magnification of the box in the left figure, showing clustered *S. aureus* (red arrows) and inflammatory cells (black arrows) (original magnification × 200).



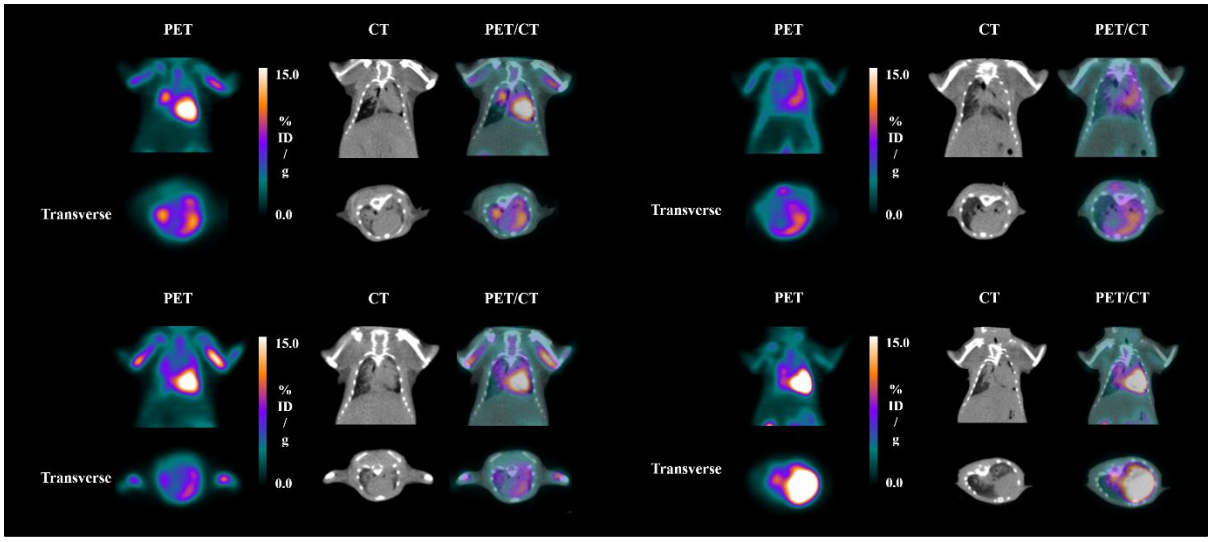
Supplementary Figure 7. MicroPET, CT, and PET/CT fusion images of ^{18}F-FDS 2 h after injection (7.4 MBq) in mice with *A. fumigatus* lung infection.



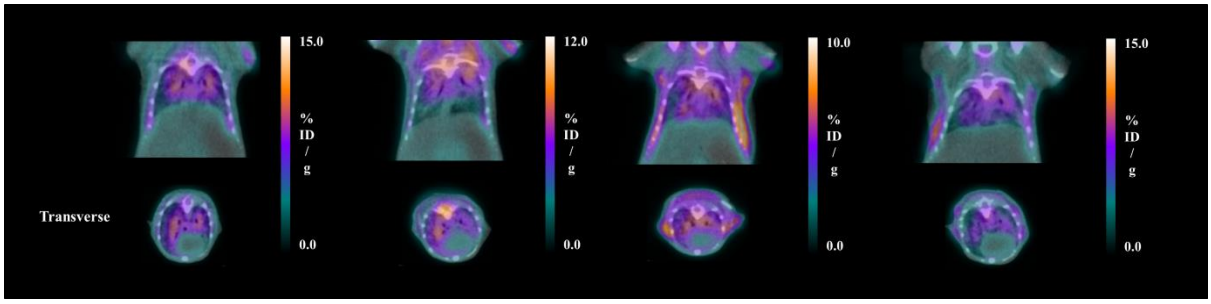
Supplementary Figure 8. (A) Autoradiographic image of ^{18}F-FDS and photograph of frozen *A. fumigatus*-infected lung sections. Mice with *A. fumigatus*-infected lungs were intravenously injected with 111 MBq of ^{18}F-FDS. The mice were sacrificed and their lungs were removed, and 25 μm frozen sections of lung were cut using a cryostat. (B) Pathological examination of *A. fumigatus*-infected lung tissue (n = 10).



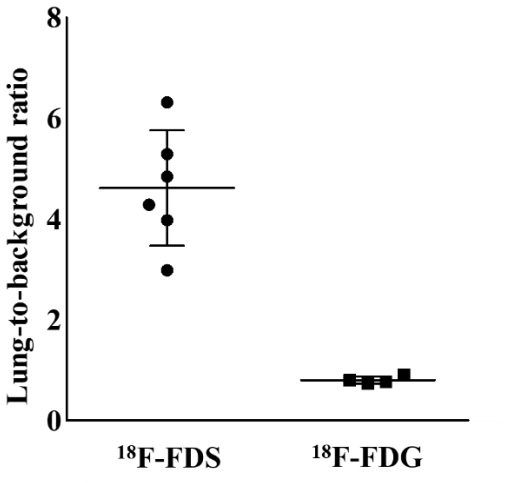
Supplementary Figure 9. MicroPET, CT, and PET/CT fusion images of ^{18}F-FDS in mice with *S. aureus*-infected lungs 2 h after injection of ^{18}F-FDS (7.4 MBq).



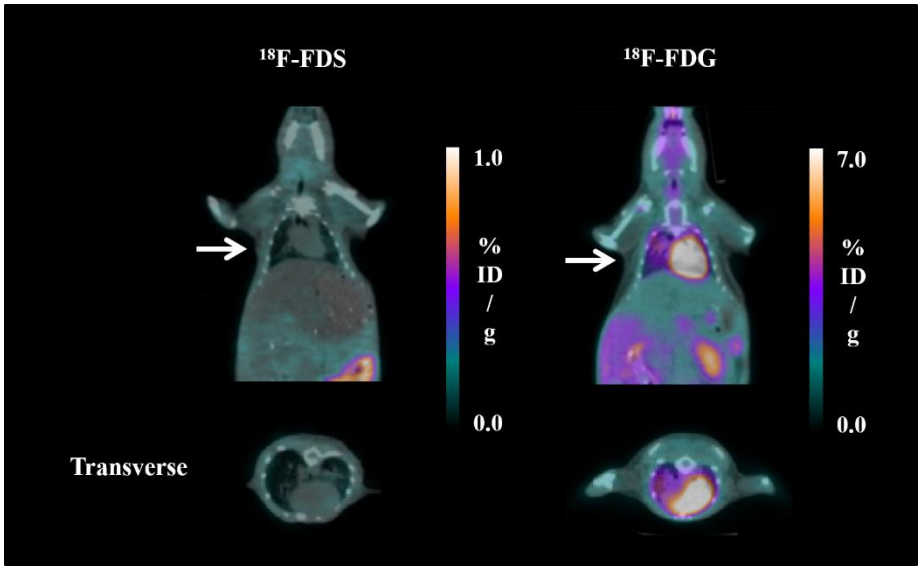
Supplementary Figure 10. MicroPET, CT, and PET/CT fusion images of ^{18}F-FDG in mice with *S. aureus* lung infection 1 h after injection of ^{18}F-FDG (7.4 MBq).



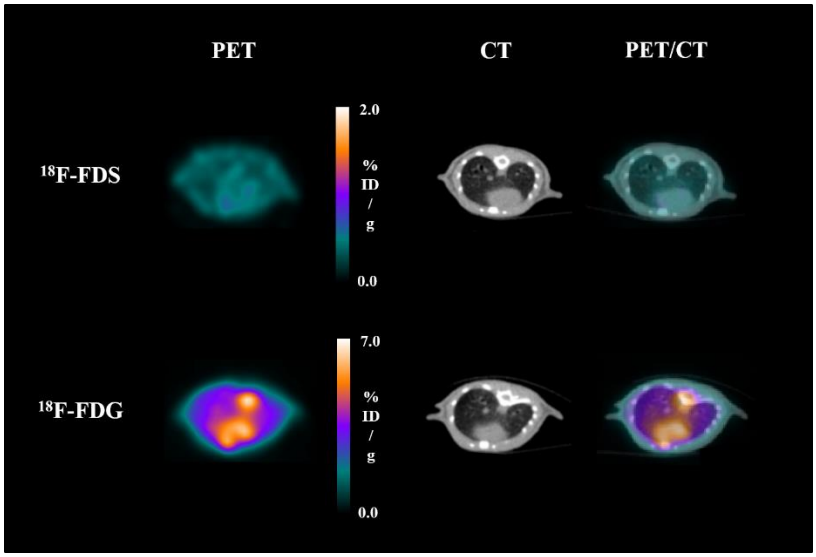
Supplementary Figure 11. MicroPET/CT fusion images of ^{18}F-FDG in mice with *A. fumigatus* lung infection 1 h after injection of ^{18}F-FDG (7.4 MBq).



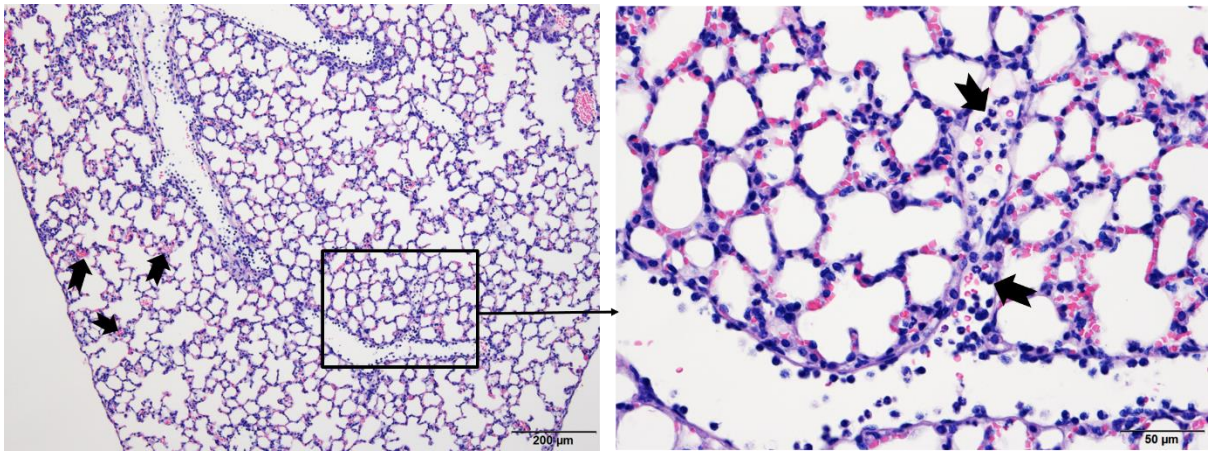
Supplementary Figure 12. Target (*A. fumigatus*-infected lung tissue)-to-background (normal muscle of hind leg) ratios of ^{18}F-FDS (2 h, n = 6) and ^{18}F-FDG (1 h, n = 4) post-injection (7.4 MBq). Data are expressed as the mean ± SD.



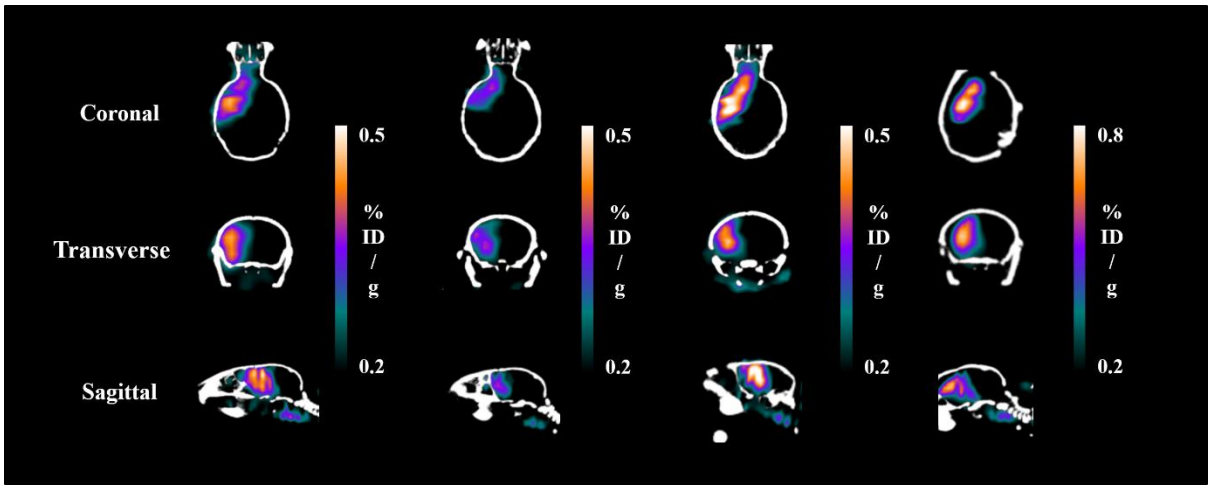
Supplementary Figure 13. MicroPET/CT fusion images of ^{18}F-FDS and ^{18}F-FDG in mice with B16F10 lung metastases (7.4 MBq).



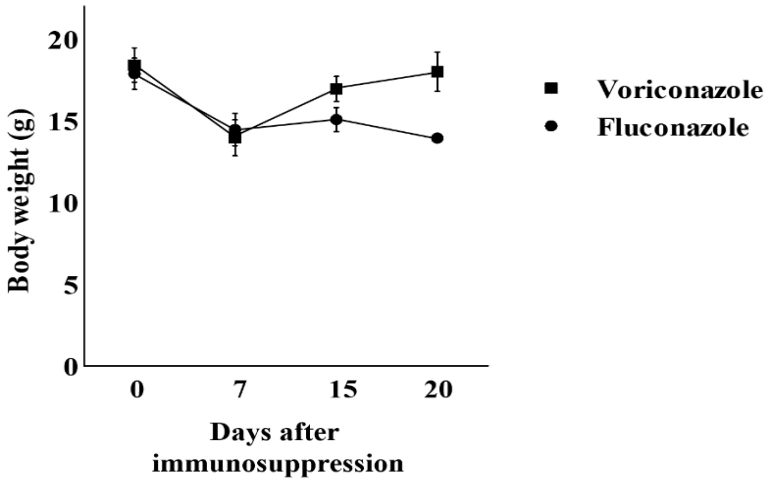
Supplementary Figure 14. MicroPET, CT, and PET/CT fusion images of ^{18}F-FDS and ^{18}F-FDG in mice with LPS-induced lung inflammation (7.4 MBq).



Supplementary Figure 15. Pathological examination of a mouse with LPS-induced acute lung injury (4 h after intraperitoneal LPS injection, n = 5). (Left) Hematoxylin and eosin (H&E) staining showing lung alveoli engorged with red blood cells (arrows) (original magnification × 100). (Right) Magnified view of the box in the left panel, showing the accumulation of neutrophils along the bronchioles.



Supplementary Figure 16. MicroPET/CT fusion images of ^{18}F-FDS in mice with *A. fumigatus* brain infection at 48 h (7.4 MBq).



Supplementary Figure 17. Body weights of *A. fumigatus*-infected mice before and after treatment with voriconazole (n = 7) or fluconazole (0 and 7 days after treatment; n = 7, 15 days after treatment; n = 5, 20 days after treatment; n = 2) for up to 20 days. Data are expressed as the mean ± SD.